4. (Amended) The method of claim 1 wherein the cell line [or retroviral particles] displays multiple growth factors.

REMARKS

Claims 1 and 4 have been amended to refer only to a retroviral packaging cell line expressing nucleic acid encoding a growth factor. Applicants submit that no new matter has been added as a result of this amendment.

Rejection of claims 10-12 under 35 U.S.C.§ 112, first paragraph

Claims 10-12 are rejected under 35 U.S.C. § 112, first paragraph for allegedly failing to enable a method for treatment of a patient by administering to the patient an effective amount of cells produced by the method of claim 1 having the nucleic acid encoding a polypeptide for treating a disease or disorder stably incorporated into their genome.

Claim 10 claims a pharmaceutical composition comprising the cells of claim 9, in combination with a pharmaceutically acceptable carrier and does not claim a method for treatment of a patient.

Claims 11 and 12 claim a method of treating a patient comprising administering to the patient an effective amount of the cells of claim 9.

The Examiner states that "the art of gene therapy is highly unpredictable" and cites the following issues regarding the invention as claimed in claims 10-12.

The Examiner states, "[y]et another problem is the quiescent state of stem cells that reduces their transduction with retroviral vectors because retroviral vectors infect only dividing cells."

Applicants submit that the claimed method of the invention is designed precisely to address this issue. Quiescent cells transformed with a retroviral packaging cell line expressing a growth factor or retroviral particles expressing a growth factor are more likely to be transformed

due to the presence of the growth factor which facilitates retroviral entry into quiescent target cells. Thus, the claimed invention is designed specifically to overcome the problem of transducing a quiescent cell. The specification teaches in examples 1-3 that pluripotent haematopoietic stem cells can be transduced using engineered retroviral packaging cells expressing surface growth factor on their surface.

The Examiner states that the claims would "encompass allogeneic, xenogeneic as well as autologous transplantation of stem cells as well as other cells." Applicants submit that in view of what was known in the art at the time of filing, one of ordinary skill in the art would, therefore, preferably use the cells of the claimed invention in autologous transplantation protocols. However, the specification does teach a method of reducing the immunogenicity of the transplanted cells that has been used successfully in allogeneic transplants. It is stated at page 9, lines 18-24, "[a]lternatively or additionally, the immunogenicity of the packaging cells can be reduced by expressing an immunosuppressive factor such as FAS-ligand on the cell surface which can bind to activated T-cells FAS receptors, triggering the T-cells to die by apoptosis. FAS-ligand expressing allogeneic cell implants have previously been shown to resist immune mediated rejection."

Applicants submit that one of skill in the art would reasonably predict that the cells of the claimed invention could be used successfully in allogenic, xenogeneic or autologous transplantation protocols. Applicants are not required to provide examples of successful allogeneic, xenogeneic or autologous transplants using the cells of the claimed invention.

The Examiner states that "the method will be complicated by the effect of the growth factor or other protein to be produced in the cell and what would have been the effect of interaction of such a factor on the immunological reaction/rejection of the administered cells."

The Examiner also states, "[t]he specification does not provide any evidence or guidance whether the stem cells expressing flt3 ligand or stem cell factor would have entered the terminal differentiation pathway."

The Examiner states further, "[t]he specification does not provide any example or any evidence or disclosure about the gene expression levels of the candidate therapeutic gene or

growth factor when the cells are transplanted in a patient".

Applicants submit further that the function of the growth factor is to facilitate retroviral entry into quiescent target cells, thereby enabling the transfer of a therapeutic gene, contained within the retroviral vector, with the aim of treating disease. The growth factor is not part of the vector genome and would therefore not be transferred to successfully transduced target cells.

In view of the above, Applicants submit that the specification does not teach expression of a growth factor in a transformed quiescent cell and is not required to enable the effect of expressing a growth factor in a transformed quiescent cell. Applicants submit further that because the specification does not teach expression of a growth factor in a transformed quiescent cell, applicants are not required to enable the expression levels of a growth factor expressed in a transformed cell.

Claims 10-12 do not require production of a growth factor in a cell. Furthermore, claims 10-12 do not require stem cells expressing flt3 ligand or stem cell factor. Thus, applicants submit that it is inappropriate for a 35 U.S.C. § 112, first paragraph rejection to be based on an element or elements that are not required by the claims.

The specification teaches a p.7, lines 19-21 and 24-35, introducing into a patient a protein or polypeptide that a patient is unable to synthesise or does not synthesise in the usual amount". Thus, the specification teaches introduction of a protein that is a normal cellular component but is absent from, or is altered in the patient, wherein the function and effect of expressing that protein in a normal cell is known. Since the function and effect of the protein in a normal cell are known, one of skill in the art would reasonably predict that the function and effect of the protein in the patient cell would be identical. In fact, that prediction underlies the invention wherein the invention provides for treating a disease by introducing a population of cells expressing a protein for treating the disease.

Regarding the issue of a sufficient level of expression of the introduced polypeptide, raised by the Examiner, a sufficient level of expression of a protein introduced into a cell according to the method of the invention will likely vary with each protein and each disorder being treated. Applicants are not required to disclose a sufficient level of all proteins that can be

used according to the claimed invention, for disease treatment. Levels of p47-*phox* protein or gp91-*phox* protein that could be used to treat chronic granulomatous disease (CGD) in a gene transfer method were known in the art as of the filing date of the instant application (Roos, 1994, Immunol. Rev., 138:121-157, Exhibit A). It is stated in the specification at page 7, line 37-page 8, line 5, "[d]iseases that might be treated using the methods and materials described herein include all forms of chronic granulomatous disease (CGD), all forms of severe combined immunodeficiency (SCID)". Applicants submit that in view of what was known in the art at the time of filing, one of skill in the art would have reasonably predicted the level of a polypeptide introduced in a cell would be useful for disease treatment.

The specification states at page 7, "introducing into a patient a protein or polypeptide that a patient is unable to synthesise or does not synthesise in the usual amount". This statement teaches that a therapeutically effective level of a protein is any level of expression of a protein introduced into a patient that lacks the protein, or a level of expression of a protein introduced into a patient that is greater than the current level in the patient, is useful according to the invention. The specification teaches how to determine the level of a polypeptide introduced into a cell. Applicants teach methods of determining the level of expression of a polypeptide introduced into a population of quiescent cells (for example, at page 15, lines 21-27, wherein a method of staining cells to measure β-galactosidase expression is described).

The specification also describes the therapeutic effects that would be observed following transplantation of the claimed cells of the invention into a patient with a particular disease. The specification teaches at p. 8, lines 14-23, "[o]ther applications of the invention include the genetic modification of haematopoietic stem cells to repopulate the immune system with genetically modified T-lymphocytes that resist HIV, the genetic modification of haematopoietic stem cells to repopulate bone marrow with haematopoietic progenitors that resist the myelosuppressive effects of cytotoxic chemotherapy, and the genetic modification of T-lymphocytes with chimeric T-cell receptors to target cytotoxic T-cells against tumours or virally infected cells."

The specification also teaches at p.27, lines 15-25, "this method is applicable for gene

therapy of inherited haematopoeitic disorders, such as the immunodeficiencies, but it could also be applicable to conditions such as haemophilia, or other conditions requiring the synthesis of a pharmacologically active compound normally present in the serum. There are also potential applications in the field of cancer therapy, primarily as a way of protecting cells from cytotoxic agents or radioprotecting them, thus giving them a survival advantage over non-treated bone marrow cells."

Thus, the specification teaches that a therapeutically effective amount of protein can be determined by monitoring the physiological changes recited above.

In view of the above, applicants submit that the specification teaches how to produce a cell that expresses an art accepted level of a protein that is known to be useful for disease treatment.

The Examiner states that in a review of the current state of the art of gene therapy, W.F. Anderson states, "[e]xcept for anecdotal reports of individual patients being helped, there is still no conclusive evidence that a gene therapy protocol has been successful in the treatment of a human disease.".

Applicants submits that it is known in the literature that ADA-SCID can be successfully treated with gene therapy (for example, Blaese et al., supra, Exhibit B; Bordignon et al., 1995, Science, 270:470-475, Exhibit C).

The specification provides support for claims to a method of treating a patient comprising administering an effective amount of the cells of claim 9. The specification teaches polypeptides useful for disease treatment according to the invention at p. 7, lines 19-21 and 24-35 wherein it is stated, "[t]ypically, the desired protein or polypeptide will be one that a patient is unable to synthesise in his or her body or does not synthesise in the usual amount...However, the concepts described herein are applicable to situations in which the nucleic acid encodes a protein or polypeptide that binds a substance that is overexpressed in a patient's body, e.g. causing some harmful physiological effect, or a protein or polypeptide that can bind to a polypeptide that is produced in a patient's body in an inactive form to activate it or in an active form to inactivate it. Preferably, the use of the present invention in these applications has the advantage that the

therapy provided by transfecting the stem cells is long lasting or permanent, thereby helping to avoid the need for frequently repeated treatment."

The specification also discloses diseases that can be treated according to the claimed methods at p.7, line 37-p.8, line 12). The specification also teaches how the claimed invention can be used for treating AIDS, cancer and viral infection at p.8, lines 14-23.

In view of all of the above, applicants respectfully request withdrawal of the 35 U.S.C. §112, first paragraph rejection of claims 10-12.

Rejection of claims 1,2, 4-5 and 7-9 under 35 U.S.C. §102(e)

Claims 1, 2, 4-5 and 7-9 are rejected under 35 U.S.C. 102(e) as being anticipated by Paul et al., U.S. Patent No. 5,736,387.

Claim 1 and dependent claims 2, 4-5 and 7-9 have been amended to claim "a method of transforming a population of quiescent cells with a nucleic acid encoding a polypeptide for treating a disease or disorder, the method comprising: exposing the cells to a retroviral packaging cell line expressing nucleic acid encoding a growth factor, so that the growth factor is displayed on the surface of the cell line, the cell line carrying a vector comprising the nucleic acid encoding the polypeptide for treating the disease or disorder, wherein the surface bound growth factor induces the cells to divide, so that the nucleic acid encoding the polypeptide for treating a disease or disorder can incorporate into the genome of the cells."

The Examiner states that Paul et al. teach envelope fusion vectors comprising chimeric targeting proteins that alter the host range of the vector, and contain a ligand moiety that binds to receptors present on target cells and an uptake moiety capable of promoting entry of the vector into the target cell. The Examiner states further that Paul et al. teach packaging cells transfected with these vectors and retroviral particles produced by these cells. The Examiner also states that the fusion protein of the Paul et al. invention can modulate the target cells in accordance with the activity of the ligand moiety (e.g. a cytokine). The Examiner also states that ligand moieties derived from flk2 ligand can be used to direct infection to lymphohematopoietic progenitor cells.

Applicants submit that Paul et al. teach chimeric target proteins capable of specifically altering the host range of retroviral vectors, comprising a ligand moiety that binds to receptors present on the target cells and an uptake moiety capable of promoting entry of a vector encoding the chimeric protein into the target cell. Paul et al. also teach methods of infecting cells with viral supernatants expressing the chimeric target proteins.

Applicants submit that Paul et al. teach retroviral vectors bearing chimeric targeting proteins on their surfaces but **do not teach retroviral packaging cells bearing chimeric targeting proteins on their surface**. (Emphasis added) A "retroviral vector" is defined in Paul et al. at column 8, lines 45-50 as, "an infectious retroviral particle comprising RNA having cis-acting packaging sequences (i.e. 'vector RNA' as defined herein), proteins encoded by the gag and pol genes, and outer surface proteins involved in binding to and entry of mammalian cells."

Claim 1 and dependent claims 2, 4-5 and 7-8 claim a method of transforming a population of **quiescent cells** with a nucleic acid encoding a polypeptide for treating a disease or disorder. Claim 9 claims a population of cells produced by the method of claim 1 having the nucleic acid encoding a polypeptide for treating a disease or disorder stably incorporated into their genome. It is stated in the specification of the instant application at p.5, lines 12-14, "[i]n this application, 'quiescent' refers to **cells that are unlikely to enter mitosis within the next 24 hours in the absence of appropriate growth stimulus**". (Emphasis added)

Applicants submit that Paul et al. do not teach quiescent cells as defined by applicant's specification, e.g., unlikely to enter mitosis within the next 24 hours in the absence of appropriate growth stimulus. Applicants submit further that Paul et al. do not teach a method of transforming a population of quiescent cells, as claimed in claims 1, 2, 4-5 and 7-8 or a population of cells produced by the method of claim 1, as claimed in claim 9. Paul et al. disclose cell types useful according to the claimed invention, including "lymphohematopoietic progenitor cells", which are defined at column 7, lines 56-65 as "cells which are typically obtained from the bone marrow or peripheral blood and **which are capable of giving rise, through cell division,** to any mature cells of the lymphoid or hematopoietic systems. This term includes committed progenitor cells with significant though limited capacity for self-renewal, as well as the more

primitive cells capable of forming spleen colonies in a CFU-S assay, and still more primitive cells possessing long-term, multilineage re-populating ability in a transplanted mammalian host." (Emphasis added).

Although Paul et al. teaches that lymphohematopoietic cells are useful according to the invention, Paul et al. do not teach lymphohematopoietic cells that are in a quiescent state. Paul et al. do not specifically teach quiescent cells, a method of transforming a population of quiescent cells or a population of transformed quiescent cells.

It is known the art that not all hematopoietic stem cells are quiescent cells (see e.g. Yu, J. Formos Med Assoc, 1996, 95:281 (Exhibit D) and Uchida et al., Curr Opin Immunol., 1993, (Exhibit E).

Uchida et al. teach that cell populations that have been isolated from adult bone marrow and are highly enriched in multipotent hematopoietic stem cells (Thy-1.1^{lo} Lin-Sca-1^{+} cells) are a "heterogeneous mixture of quiescent and self-renewing hematopoietic stem cells as well as multi-lineage expanding cells."

Yu teach that hematopoietic cells are not always quiescent and disclose two populations of stem cells: actively cycling (e.g. undergoing mitosis) and non-cycling.

Thus, these references teach that hematopoietic stem cells are not always in a quiescent state. That is, the term hematopoietic stem cell is not synonymous with the term quiescent. Thus, in the absence of a specific teaching of quiescent cells, it is unclear if any of the cells taught in Paul et al. are in a quiescent state.

Claims 1, 2, 4-5 and 7-9 all claim " a method of exposing the cell to **a retroviral packaging cell line expressing nucleic acid encoding a growth factor**, so that the growth factor is displayed on the surface of the cell line...". (Emphasis added)

Thus, claims 1, 2, 4-5 and 7-9 refer to transforming quiescent cells with a retroviral packaging cell line expressing nucleic acid encoding a growth factor.

Applicants submit that Paul et al. do not teach a retroviral packaging cell line expressing a nucleic acid encoding a growth factor but rather teach only retroviral particles "bearing

chimeric targeting proteins on their surface."

In view of the above, applicants submit that Paul et al. do not teach a method of transforming a population of **quiescent** cells with a nucleic acid encoding a polypeptide for treating a disease or disorder, as claimed in claims 1, 2, 4-5 and 7-8 or a population of cells produced by the method of claim 1, as claimed in claim 9.

Applicants submit further that Paul et al. do not teach a "method of transforming a population of **quiescent** cells with a nucleic acid encoding a polypeptide for treating a disease or disorder, the method comprising: exposing the cells to a retroviral packaging cell line expressing nucleic acid encoding a growth factor, so that the growth factor is displayed on the surface of the cell line..." as claimed in claims 1, 2, 4-5 and 7-8.

In view of the above, applicants respectfully request withdrawal of the 35 U.S.C. §102(e) rejection over Paul et al.

Rejection of claim 3 under 35 U.S.C. §103(a)

Claim 3 is rejected for alleged obviousness over Paul et al. in view of Lyman et al. (U.S. Patent 5,554,512).

The Examiner states that Lyman et al. teach ligands for flt3 receptors that are capable of inducing the growth, proliferation and differentiation of progenitor and stem cells, and DNA encoding flt3 ligands. The Examiner also states that Lyman et al. assert that the flt3 ligand of the invention is useful for disease treatment and can mobilize the number of circulating peripheral blood progenitor cells or stem cells. The Examiner also states that Lyman et al. teach that a cDNA encoding flt3 ligand may be transfected into cells to deliver its gene product to the targeted cell or tissue.

The Examiner concludes that at the time of the invention, it would have been obvious to one of ordinary skill in the art to modify the retroviral vector of Paul (that encodes a fusion protein of a cytokine and a retroviral envelope protein) by cloning into it the flt3 ligand cDNA taught by Lyman et al., transfect the vector into cells to produce packaging cells that would

produce retroviral particles capable of infecting stem cells and affect their proliferation due to the expression of the flt3 ligand. The Examiner states that the motivation for combining Paul et al. and Lyman et al. is found in the teachings of Lyman et al. that flt3 ligand can be used to stimulate the proliferation of stem cells, and the teachings of Paul et al. that the fusion protein can be used for expression of cytokines that stimulate proliferation of progenitor cells.

Applicants submit that Lyman et al. teach flt3 ligands, DNA encoding flt3 ligand, cells expressing a vector encoding flt3 ligand, antibodies that bind flt3 ligand, the generation of transgenic mice overexpressing flt3 ligand, a method of using flt3 ligand in peripheral stem cell transplantation and a method of purifying a hematopoietic progenitor using flt3 ligand. Lyman et al. does not teach quiescent cells or a method of transforming a population of quiescent cells. It is stated in Lyman et al. at column 6, line 61-column 7, line 1,

> "[t]he method of the invention described above optionally comprises a subsequent in vivo procedure comprising administering flt3-L alone or in sequential or concurrent combination with an engraftment growth factor to a patient following transplantation of the cellular preparation to facilitate engraftment and augment proliferation of engrafted hematopoietic progenitor or stem cells from the cellular preparation."

However, Lyman et al. do not teach quiescent hematopoietic progenitor or stem cells. As stated above, hematopoietic cells or stem cells are not always quiescent. Thus, in the absence of a specific teaching of quiescent hematopoietic or stem cells, it cannot be assumed that the hematopoietic progenitor or stem cells of the Lyman et al. invention are in a quiescent state.

Applicants submit further that Lyman et al. do not teach a retroviral packaging cell line expressing nucleic acid encoding a growth factor, as claimed in claim 3.

Even if the references are combined, they do not provide the invention as claimed.

Applicants submit that even if Paul et al. is combined with Lyman et al., the two disclosures do not provide the invention as claimed in claim 3. That is, the recited combination lacks essential elements of the claimed invention.

The recited combination of references does not disclose a method of transforming a population of quiescent cells with a nucleic acid encoding a polypeptide for treating a disease or disorder, the method comprising: exposing the cells to a retroviral packaging cell line expressing nucleic acid encoding a growth factor, wherein the growth factor is stem cell factor (SCF) or FLT3 ligand, as claimed in claim 3.

The method of transforming a population of **quiescent** cells comprising exposing the cells to a **retroviral packaging cell line expressing nucleic acid encoding a growth factor**, as claimed in claim 3 is unobvious over Paul et al. and Lyman et al. because neither of these two disclosures teach or suggest a method of transforming a population of quiescent cells or a retroviral packaging cell line expressing nucleic acid encoding a growth factor.

It is submitted that one of skill in the art would not be able to combine the retroviral vector of Paul et al. with the flt3 ligand cDNA taught by Lyman et al. and produce a retroviral packaging cell line expressing nucleic acid encoding a growth factor that can infect quiescent cells and affect their proliferation, so as to arrive successfully at the invention as described in the instant claim because neither of Paul et al. or Lyman et al. or their combination teaches or suggests the essential element of the claim.

In view of all of the above, applicants request withdrawal of the 35 U.S.C. §103(a) rejection over the combination of Paul et al. and Lyman.

Rejection of claim 6 under 35 U.S.C. §103(a)

Claim 6 is rejected for alleged obviousness over Paul et al., Lyman et al. and further in view of Beutler et al. (U.S. Patent 5,447,851).

The Examiner states that Beutler et al. teach a DNA encoding a chimeric protein comprising the extracellular domain of a receptor fused to IgG and a linker peptide present at a site within the chimeric protein which can be cleaved to separate the two proteins of the fusion protein.

The Examiner also states that at the time of the invention, it would have been obvious to

one of ordinary skill in the art to modify the vector of Paul et al. to include sequence encoding a cleavable peptide polylinker between the sequences encoding the growth factor and the envelope protein with a reasonable expectation of success because Beutler et al,. disclose the method and sequence incorporating the linker in the fusion protein. The motivation to introduce the linker in the fusion protein would be to allow cleavage of the growth factor from the envelope protein to facilitate the action of the growth factor on the physiology of the infected cell.

Applicants submit that Beutler et al. teach construction of a chimeric cytokine receptor reagent comprising an extracellular portion of a cytokine receptor polypeptide attached through oligomers encoding cleavable peptide linkers to a sequence encoding portions of IgG heavy chain polypeptides. Beutler et al. also teach a DNA sequence encoding this chimeric reagent, and cells transfected with a vector encoding this chimeric reagent. Further, Beutler et al. teach the use of the cytokine receptor chimeric reagent for birth control and as an anti-tumor agent for placental tumors. Beutler et al. do not teach quiescent cells or a method of transforming a population of quiescent cells, as claimed in claim 6.

Beutler et al. do not teach a retroviral packaging cell line expressing nucleic acid encoding a growth factor or a method of transforming a population of quiescent cells comprising exposing the cells to a retroviral packaging cell line expressing nucleic acid encoding a growth factor, as claimed in claim 6.

No motivation to combine the references.

The Examiner has stated that the motivation to combine Paul et al. and Lyman et al. and Beutler et al. is to facilitate the action of the growth factor on the physiology of the infected cells by allowing cleavage of the growth factor from the envelope protein.

Applicants submit that Beutler et al. teach cleavage of a chimeric protein after the protein has been secreted into the cellular supernatant. Beutler et al. do not teach or suggest intracellular cleavage of a chimeric protein. Applicants submit further that there would be no motivation to combine Paul et al., Lyman et al. and Beutler et al. to create a cleavable chimeric protein wherein cleavage would occur in a cell that has been infected according to the method of Paul et al. since

there is no reason to expect that the addition of a cleavage agent to a cell would not interfere with the normal physiology of the cell or with the effects of the cytokine portion of the chimeric protein being introduced into the cell.

In view of the above, Applicants submit that there is no motivation to combine Paul et al., Lyman et al. and Beutler et al., and requests withdrawal of the 103(a) rejection over this combination of references.

Even if the references are combined, they do not provide the invention as claimed.

Applicants submit that even if Paul et al. is combined with Lyman et al., and Beutler et al. the three disclosures do not provide the invention as claimed in claim 6. That is, the recited combination lacks essential elements of the claimed invention.

The recited combination of references does not disclose a method of transforming a population of **quiescent** cells with a nucleic acid encoding a polypeptide for treating a disease or disorder, the method comprising: exposing the cells to **a retroviral packaging cell line expressing nucleic acid encoding a growth factor**, wherein the growth factor is expressed as a fusion with a viral envelope protein and is fused to the envelope protein via a cleavable linker, as claimed in claim 6. (Emphasis added)

The method of transforming a population of quiescent cells, as claimed in claim 6, is unobvious over Paul et al., Lyman et al. and Beutler et al. because none of these three disclosures teach or suggest a method of transforming a population of quiescent cells with a retroviral packaging cell line expressing nucleic acid encoding a growth factor.

It is submitted that one of skill in the art would not be able to combine the retroviral vector of Paul et al. with the flt3 ligand cDNA taught by Lyman et al., and the cleavable peptide polylinker of Beutler et al. introduced between the sequences encoding the growth factor and the envelope protein, and produce a retroviral packaging cell line expressing nucleic acid encoding a growth factor, that can infect stem cells and affect their proliferation, so as to arrive successfully at the invention as described in the instant claim because none of Paul et al., Lyman et al.,

Beutler et al. or their combination teaches or suggests the essential element of the claim.

In view of all of the above, applicants request withdrawal of the 35 U.S.C. §103(a) rejection over the combination of Paul et al., Lyman et al. and Beutler et al.

Applicants submit that in view of all of the above, the claims are patentable and a notice of allowance to that effect is respectfully requested.

Respectfully submitted,

7/6/00
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